to in situ digestion treatment and MALDI matrix deposition. The SPRi sensor surface was inserted directly in an appropriate MALDI plate holder and MALDI-MS and MS/MS complete identification of the retained antigens was performed directly from each individual spots on the biochip. Using this process, the transfer of the biochip into the MALDI apparatus consecutive to a SPR imaging experiment was straightforward without intermediate treatment that could lead to sample loss and/or contaminations.
A same surface array is dedicated to SPRi for affinity separation and subsequent MALDI-MS and MS/MS identification of the retained ligands from complex solution.

1258-Pos
Thermodynamic Principles of Metal Binding to Biological Systems
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We study the selective binding of the softer K+ ion over the harder Na+ and Mg++ ions to the 58 nucleotide ribosomal RNA fragment and the S2 site of the KcsA K+ channel using all atom MD simulations. The ribosomal RNA is interesting in that it binds the K+ over the harder Mg++ ion. We calculate the free energy of exchanging K+ with Mg++ in the binding site relative to the change in the aqueous solution. In order to explain the selectivity, we find that it is necessary to consider the change in the internal energy of the ion binding site. Interestingly, similar physics is observed in the KcsA S2 site. There we elucidate the role of coordination number fluctuations, ion-site binding energetics, and site-site interaction energetics in determining the selectivity of the S2 site for K+: once again, the reduced strain in the site in the presence of K+ (relative to Na+) explains selectivity. Thus, despite the uncommon origin and completely different chemical composition of the binding site, in both the cases the binding site is constricted in the presence of the harder ion and causes an increase in the unfavorable electrostatic strain. This strain is what leads to the observed selectivity for the softer K+ ion.

1259-Pos
Effect of Variants at the 78th Residue on Neocarzinostatin Chromophore Release
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Neocarzinostatin is a potent antitumor antibiotics chromoprotein complex. It consists of a labile enediyne chromophore and a protective carrier protein. Release of the bioactive chromophore is the initial key step in its cytotoxic mode of action. We showed that F78 of the carrier protein plays an important role in gating the release. To further study the structural role of 78th side chain in the release mechanism, we made variants at the 78th residue using a PCR-based *in vitro* mutagenesis method. The identity and purity of the expressed protein variants were confirmed by SDS-PAGE, UV, HPLC, and MS spectroscopy. Oxygenation was applied to promote formation of disulfide bonds when necessary, and the integrity of disulfide linkages was verified by a designed iodoacetamide-based test. Thermal denaturation study and CD spectroscopic analysis showed that stability and backbone conformation of these variants were conserved as compared to the wild-type protein. Two-dimensional ^{15}N-^{1}H HSQC NMR study suggested that the majority of residues around the binding cleft were not disturbed. Reconstitution of the enediyne chromophore into these protein variants showed efficient binding and the binding structure appeared to be similar to that of the native neocarzinostatin. Once the backbone structural effect was excluded, side chain structural effect on kinetic release was studied by monitoring fluorescence changes on samples containing glutathione and the reconstituted variants. The results showed that, except aromatic side chains, the basic variants increased the release rate more significantly than the acidic ones. Depended upon the length, steric hindrance, or hydrophobicity, the variants with aliphatic side chain also increased the release rate at different extent.

1260-Pos
Mechanical Regulation of a Cell Binding Site: Bacterial Adhesins can Distinguish Different Physical States of Fibronectin
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Using extracellular matrix (ECM) fibers, we provide an experimental proof that mechanical forces can disrupt a bacterial binding site. Several bacterial species (gram positive and spirochetes, e.g. S. Aureus and B. Burgdorferi) express membrane-anchored fibronectin (Fn) binding proteins composed of multiple Fn-binding repeats (FnBRs), each of which bridges several Fn type I modules (FnI). As demonstrated here, the repetitive design of bacterial adhesins enables them to recognize the physical state of a multimodular protein: stretching fibrillar fibronectin causes a structural mismatch switching this multivalent binding site to low affinity as revealed at atomic resolution by steered molecular dynamics and confirmed utilizing a FRET-based Fn fiber stretching assay. The application of external mechanical force on the termini of Fn leads to an increase of the intermodular distance between the adjacent FnI modules, and the peptide can no longer bind via multivalent interactions. Even phylogenetically distinct bacteria use similar motifs to bind fibronectin, but have different numbers of FnBRs.

1261-Pos
Quantifying Water-Mediated Protein-Ligand Interactions in a Glutamate Receptor
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It is becoming increasingly clear that careful treatment of water molecules in ligand-protein interactions is required in many cases if the correct binding pose is to be identified in molecular docking. Water can form complex bridging networks and can play a critical role in dictating the binding mode of ligands. A particularly striking example of this can be found in the ionotropic glutamate receptors. Despite possessing similar chemical moieties, crystal structures of glutamate and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) in complex with the ligand-binding core of the GluA2 ionotropic glutamate receptor revealed, contrary to all expectation, two distinct modes of binding. The difference appears to be related to the position of water molecules within the binding pocket. However, it is unclear exactly what governs the preference for water molecules to occupy a particular site in any one binding mode. In this work we use density functional theory (DFT) calculations to investigate the interaction energies and polarization effects of the various components of the binding pocket. Our results show i) that the ligand and its binding mode dictate the interaction energy of a key water molecule which can be thought of as part of the ligand rather than part of the protein ii) that polarization effects can be large and iii) that the interaction energy of a neighbouring water is particularly large (compared to the other waters in the binding pocket) and may offer a route to compounds with improved affinity if it can be displaced. We discuss the results within the broader context of drug-design.

1262-Pos
Epitope Mapping of Anti-DCP Antibody Using Fluorescence Correlation Spectroscopy
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Des-gamma-carboxy prothrombin (DCP), also known as protein induced by vitamin K antagonist (PIVKA-II), is a recognized clinical marker for hepatocellular carcinoma. Prothrombin contains 10 gluamic acid (Glu) residues within its N-terminus (GLA domain) which are post modified to gamma-carboxyglutamic acid (GLA). DCP is an abnormal form of prothrombin in which some of the 10 glutamic acid residues remain unmodified. A monoclonal antibody was developed to specifically recognize DCP, but not prothrombin. In this study we identified the epitope of the anti-DCP antibody using a series of short peptides representing the GLA domain. For each peptide, a single Glu residue was replaced with a GLA residue. The critical Glu residues recognized by the antibody were identified in a competitive format using fluorescence correlation spectroscopy (FCS). We also evaluated the DCP specificity of the antibody using homologues peptide from various GLA domain proteins present in various blood coagulation factors. The dissociation constant of the antibody was determined using FRET based method.

1263-Pos
The Effect of Alpha-Helix Linker Length on Volatile General Anesthetic Binding to the Four-Alpha-Helix Bundle (Aα2-L38M)2
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In order to investigate the effects of the length of the linker connecting the alpha-helices in the di-alpha-helical protein $A\alpha_2$-L38M on the overall structure and on the affinity of anesthetic binding to four-alpha-helix bundle $(A\alpha_2\text{-}L38M)_2$, we designed constructs containing six and four glycine residue linkers instead of the original eight, respectively. We used site-directed mutagenesis with primers designed to remove two and four glycine residues, respectively, out of eight residues of the original glycine linker present in the four-alpha-helix bundle $(A\alpha_2\text{-}L38M)_2$. Variant proteins were expressed in bacteria and purified to homogeneity using reverse-phase HPLC. Protein identities were verified using mass spectrometry. Our initial studies reveal that the variant four-alpha-helix bundle with a four glycine linker binds halothane with a dissociation constant of 1.00 $\pm$ 0.05 mM, as assessed using fluorescence spectroscopy. This represents a 10-fold decreased affinity for the volatile general anesthetic compared to the original $(A\alpha_2\text{-}L38M)_2$ design, which featured an eight glycine linker. The results indicate that the length of the glycine linker, and the resulting dynamic behavior of the four-alpha-helix bundle protein, dramatically influence volatile general anesthetic binding affinity.